Species Reactivity: Human/Mouse/Rat

Specificity: Detects human Vinculin in direct ELISAs and Western blots.

Source: Monoclonal Mouse IgG2A Clone # 728526

Purification: Protein A or G purified from hybridoma culture supernatant

Immunogen: E. coli-derived recombinant human Vinculin Lys1020-Gln1134

Accession #: P18206

Formulation: Lyophilized from a 0.2 μm filtered solution in PBS with Trehalose. See Certificate of Analysis for details.

*Small pack size (-SP) is supplied either lyophilized or as a 0.2 μm filtered solution in PBS.

APPLICATIONS

Please Note: Optimal dilutions should be determined by each laboratory for each application. General Protocols are available in the Technical Information section on our website.

<table>
<thead>
<tr>
<th>Recommended Concentration</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Blot</td>
<td>2 µg/mL</td>
</tr>
<tr>
<td>Immunohistochemistry</td>
<td>8-25 µg/mL</td>
</tr>
<tr>
<td>Simple Western</td>
<td>20 µg/mL</td>
</tr>
</tbody>
</table>

DATA

Western Blot

Detection of Human Vinculin by Western Blot. Western blot shows lysates of HeLa human cervical epithelial carcinoma cell line and HepG2 human hepatocellular carcinoma cell line. PVDF membrane was probed with 2 µg/mL of Mouse Anti-Human Vinculin Monoclonal Antibody (Catalog # MAB6896) followed by HRP-conjugated Anti-Mouse IgG Secondary Antibody (Catalog # HAF007). A specific band was detected for Vinculin at approximately 124 kDa (as indicated). This experiment was conducted under reducing conditions and using Immunoblot Buffer Group 1.

Immunohistochemistry

Vinculin in Human Uterus. Vinculin was detected in immersion fixed paraffin-embedded sections of human uterus using Mouse Anti-Human Vinculin Monoclonal Antibody (Catalog # MAB6896) at 15 µg/mL overnight at 4 °C. Before incubation with the primary antibody, tissue was subjected to heat-induced epitope retrieval using Antigen Retrieval Reagent-Basic (Catalog # CTS013). Tissue was stained using the Anti-Mouse HRP-DAB Cell & Tissue Staining Kit (brown; Catalog # CTS002) and counterstained with hematoxylin (blue). Specific staining was localized to smooth muscle cells. View our protocol for Chromogenic IHC Staining of Paraffin-embedded Tissue Sections.

Simple Western

Detection of Human Vinculin by Simple Western™. Simple Western lane view shows lysates of HeLa human cervical epithelial carcinoma cell line and PC-3 human prostate cancer cell line, loaded at 0.2 mg/mL. A specific band was detected for Vinculin at approximately 122 kDa (as indicated) using 20 µg/mL of Mouse Anti-Human/Mouse/Rat Vinculin Monoclonal Antibody (Catalog # MAB6896). This experiment was conducted under reducing conditions and using the 12-230 kDa separation system.

PREPARATION AND STORAGE

Reconstitution: Sterile PBS to a final concentration of 0.5 mg/mL.

Shipping: The product is shipped at ambient temperature. Upon receipt, store it immediately at the temperature recommended below.

*Small pack size (-SP) is shipped with polar packs. Upon receipt, store it immediately at -20 to -70 °C

Stability & Storage: Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 12 months from date of receipt, -20 to -70 °C as supplied.
- 1 month, 2 to 8 °C under sterile conditions after reconstitution.
- 6 months, -20 to -70 °C under sterile conditions after reconstitution.
VCL (vinculin) is a 117 kDa actin binding cytoskeletal phosphoprotein found in adherens-type cell-cell and cell-matrix junctions. The 1066 amino acid (aa) human vinculin contains an N-terminal globular head (aa 2-835) with a talin interaction domain (aa 168-208) and three tandem repeats (aa 259-589), a pro-rich linker region (aa 826-878), and a C-terminal tail that facilitates phospholipid membrane insertion (aa 1003-1134). An 1134 aa form termed metavinculin contains 68 aa inserted after aa 915 and mainly occurs in cardiac, skeletal and smooth muscle. Human, mouse and rat vinculin share 100% aa sequence identity within the region used as an immunogen.